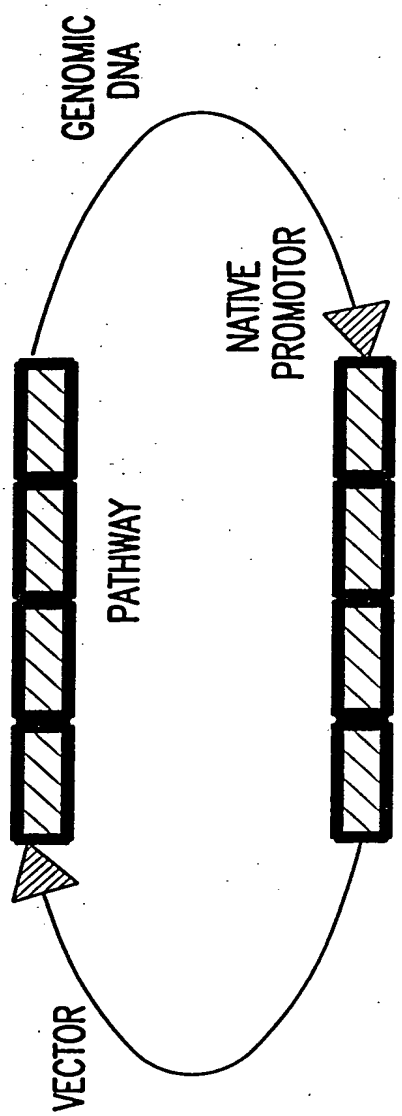

FIG.1

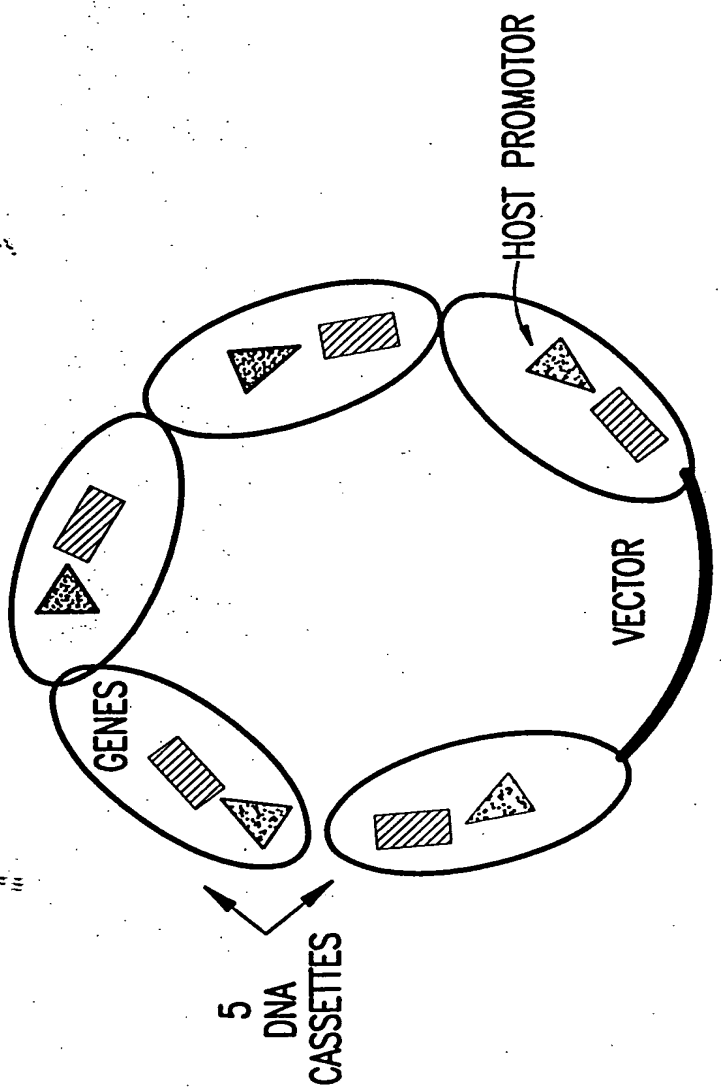

FIG.2

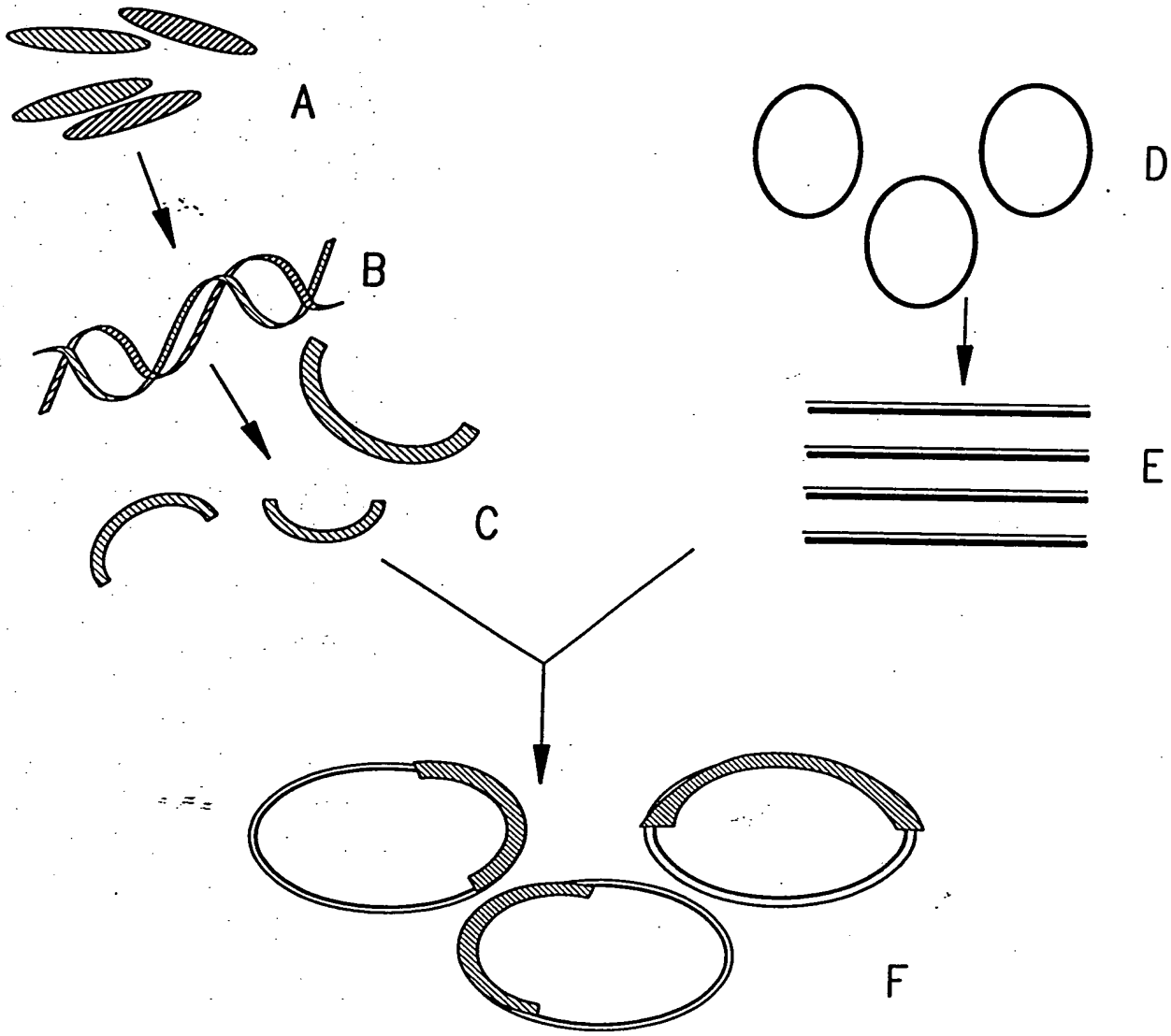

FIG.3

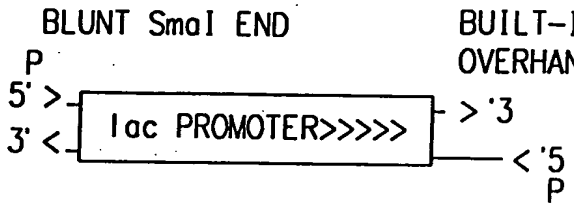

FIG.4A

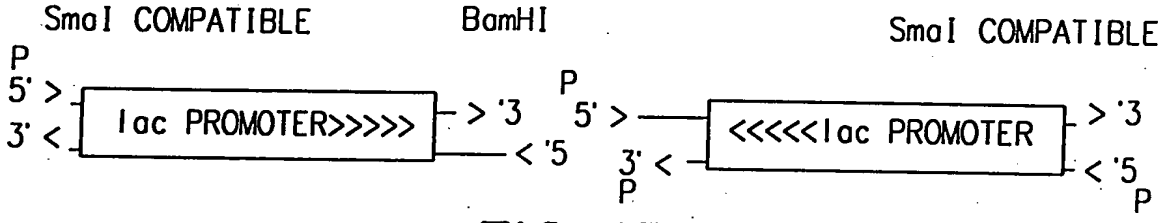

FIG.4B

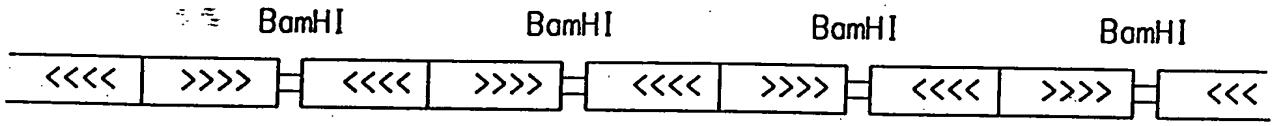

FIG.4C

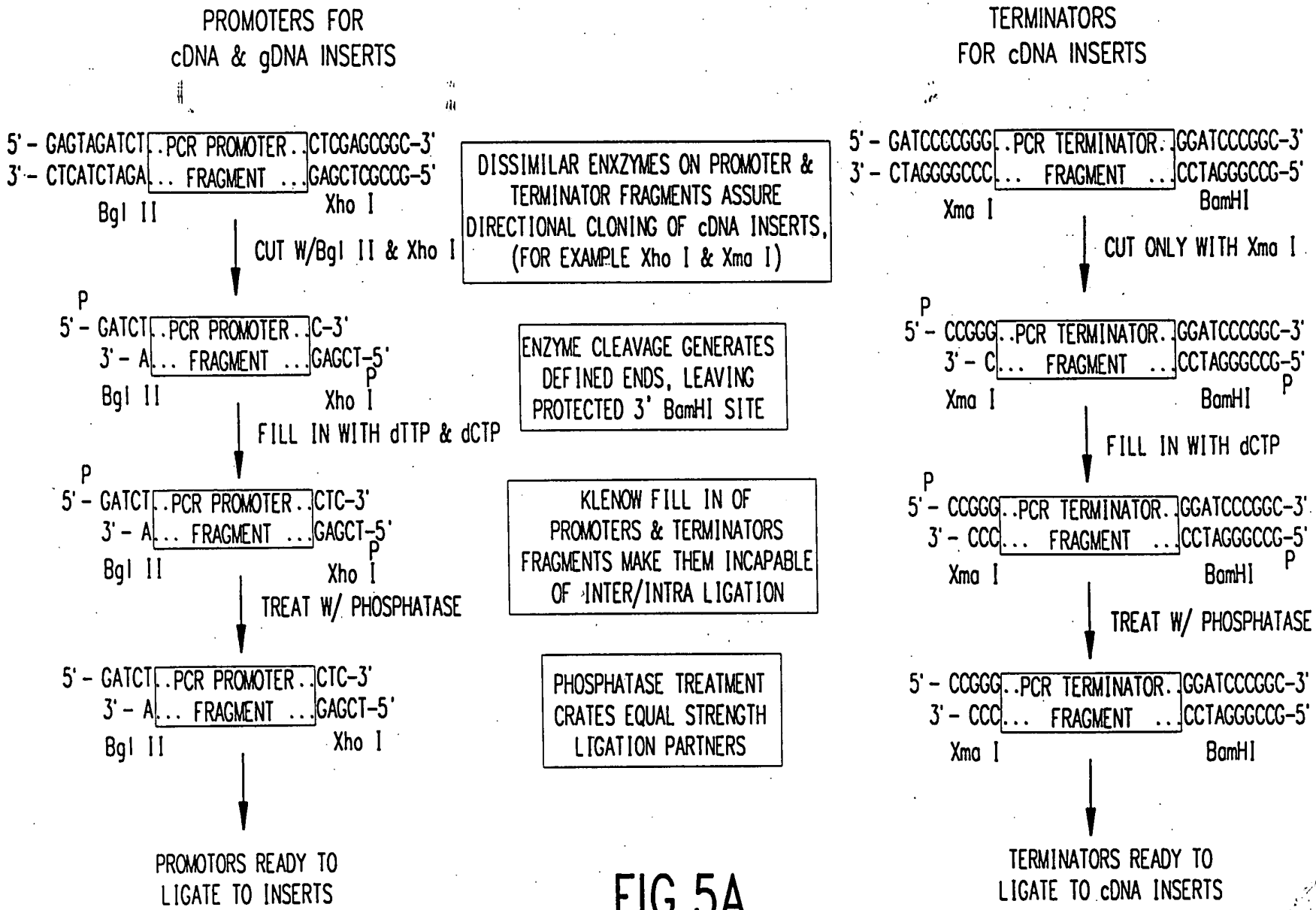
PROMOTERS FOR
cDNA & gDNA INSERTS
5' - GAGTAGATCT..PCR PROMOTER..CTCGAGCGGC-3'
3' - CTCATCTAGA... FRAGMENT ...GAGCTCGCCG-5'
Bgl II
Xho I
CUT W/Bgl II & Xho I
P
5' - GATCT..PCR PROMOTER..C-3'
3' - A... FRAGMENT ...GAGCT-5'
P
Bgl II
Xho I
FILL IN WITH dTTP & dCTP
P
5' - GATCT..PCR PROMOTER..CTC-3'
3' - A... FRAGMENT ...GAGCT-5'
P
Bgl II
Xho I
TREAT W/ PHOSPHATASE
5' - GATCT..PCR PROMOTER..CTC-3'
3' - A... FRAGMENT ...GAGCT-5'
Bgl II
Xho I
PROMOTORS READY TO
LIGATE TO INSERTS
DISSIMILAR ENXZYMES ON PROMOTER & TERMINATOR FRAGMENTS ASSURE DIRECTIONAL CLONING OF cDNA INSERTS, (FOR EXAMPLE Xho I & Xma I)
ENZYME CLEAVAGE GENERATES DEFINED ENDS, LEAVING PROTECTED 3' BamHI SITE
KLENOW FILL IN OF PROMOTERS & TERMINATORS FRAGMENTS MAKE THEM INCAPABLE OF INTER/INTRA LIGATION
PHOSPHATASE TREATMENT CRATES EQUAL STRENGTH LIGATION PARTNERS
TERMINATORS
FOR cDNA INSERTS
5' - GATCCCCGGG..PCR TERMINATOR..GGATCCCGGC-3'
3' - CTAGGGGCCC... FRAGMENT ...CCTAGGGCCG-5'
Xma I
BamHI
CUT ONLY WITH Xma I
P
5' - CCGGG..PCR TERMINATOR..GGATCCCGGC-3'
3' - C... FRAGMENT ...CCTAGGGCCG-5'
P
Xma I
BamHI
FILL IN WITH dCTP
P
5' - CCGGG..PCR TERMINATOR..GGATCCCGGC-3'
3' - CCC... FRAGMENT ...CCTAGGGCCG-5'
P
Xma I
BamHI
TREAT W/ PHOSPHATASE
5' - CCGGG..PCR TERMINATOR..GGATCCCGGC-3'
3' - CCC... FRAGMENT ...CCTAGGGCCG-5'
Xma I
BamHI
TERMINATORS READY TO
LIGATE TO cDNA INSERTS

FIG.5A

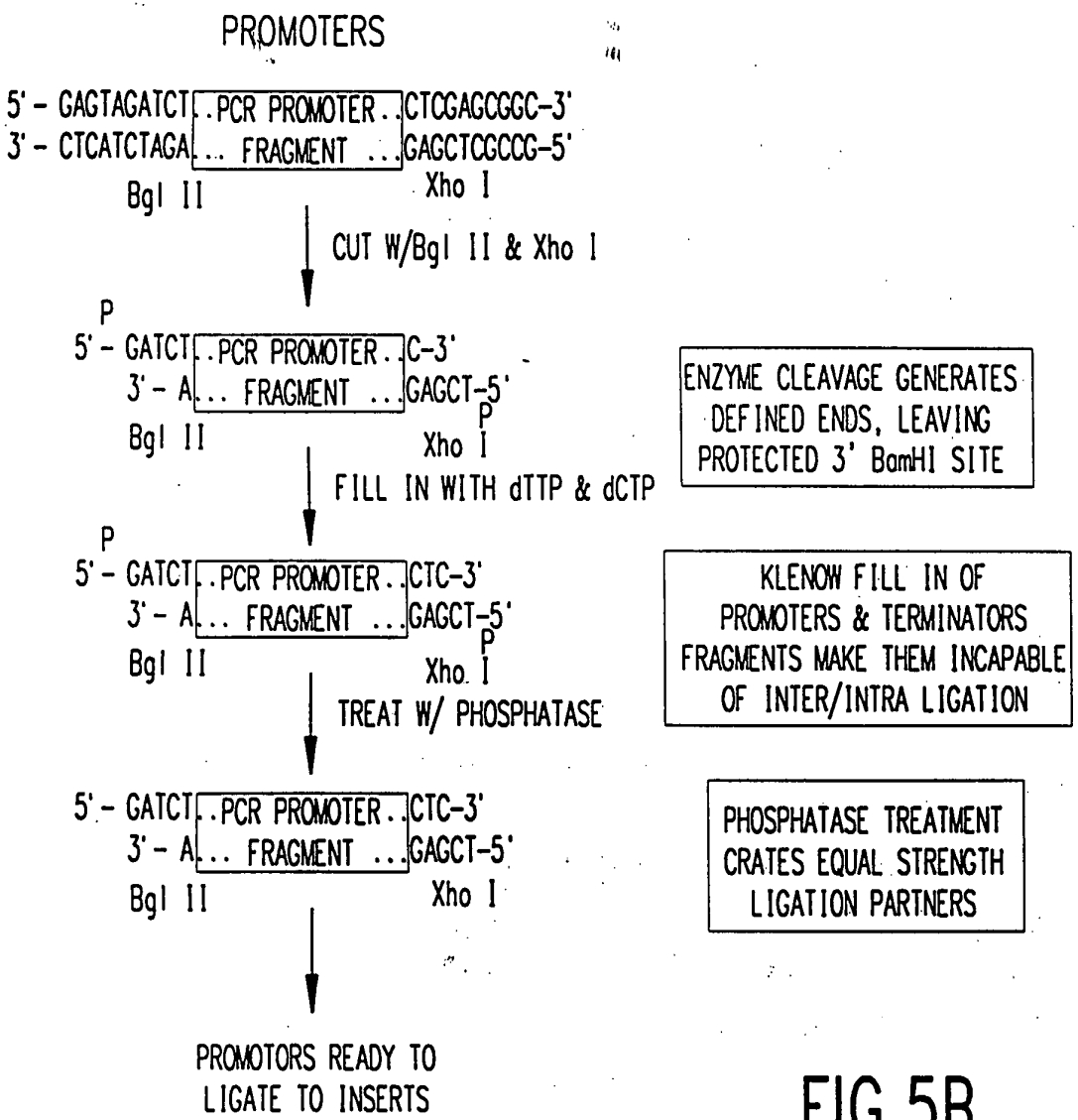

FIG.5B

8757-007

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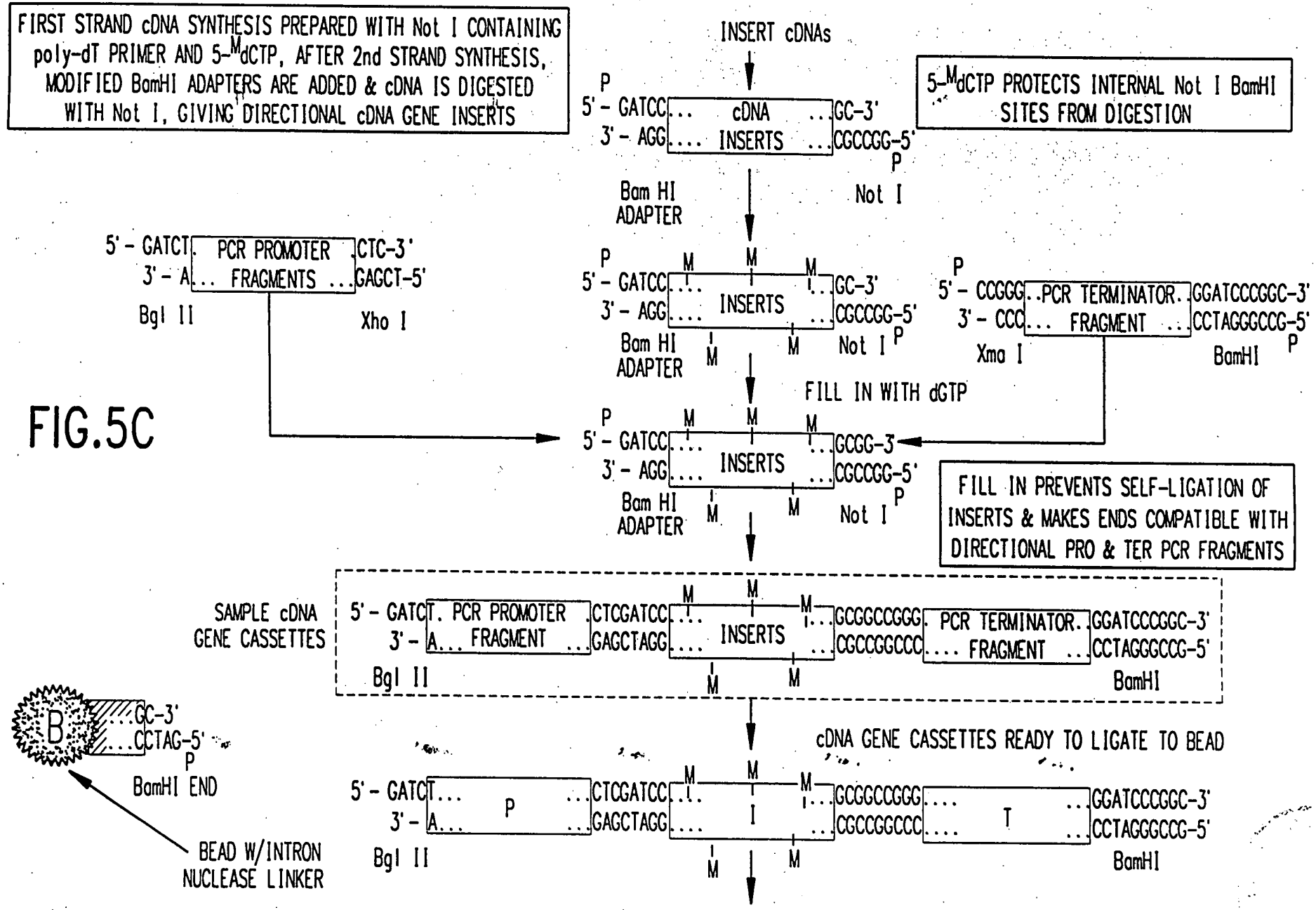

FIRST STRAND cDNA SYNTHESIS PREPARED WITH Not I CONTAINING poly-dT PRIMER AND 5-MdCTP, AFTER 2nd STRAND SYNTHESIS, MODIFIED BamHI ADAPTERS ARE ADDED & cDNA IS DIGESTED WITH Not I, GIVING DIRECTIONAL cDNA GENE INSERTS
INSERT cDNAs
5-MdCTP PROTECTS INTERNAL Not I BamHI SITES FROM DIGESTION
5' - GATCC ... cDNA ... GC-3'
3' - AGG .... INSERTS ... CGCCGG-5'
Bam HI ADAPTER
Not I
5' - GATCT PCR PROMOTER CTC-3'
3' - A ... FRAGMENTS ... GAGCT-5'
Bgl II
Xho I
5' - CCGGG PCR TERMINATOR GGATCCCGGC-3'
3' - CCC ... FRAGMENT ... CCTAGGGCCG-5'
Xma I
BamHI
FILL IN WITH dGTP
FILL IN PREVENTS SELF-LIGATION OF INSERTS & MAKES ENDS COMPATIBLE WITH DIRECTIONAL PRO & TER PCR FRAGMENTS
SAMPLE cDNA GENE CASSETTES
cDNA GENE CASSETTES READY TO LIGATE TO BEAD
B
BamHI END
BEAD W/INTRON NUCLEASE LINKER

FIG.5C

FIG.5D

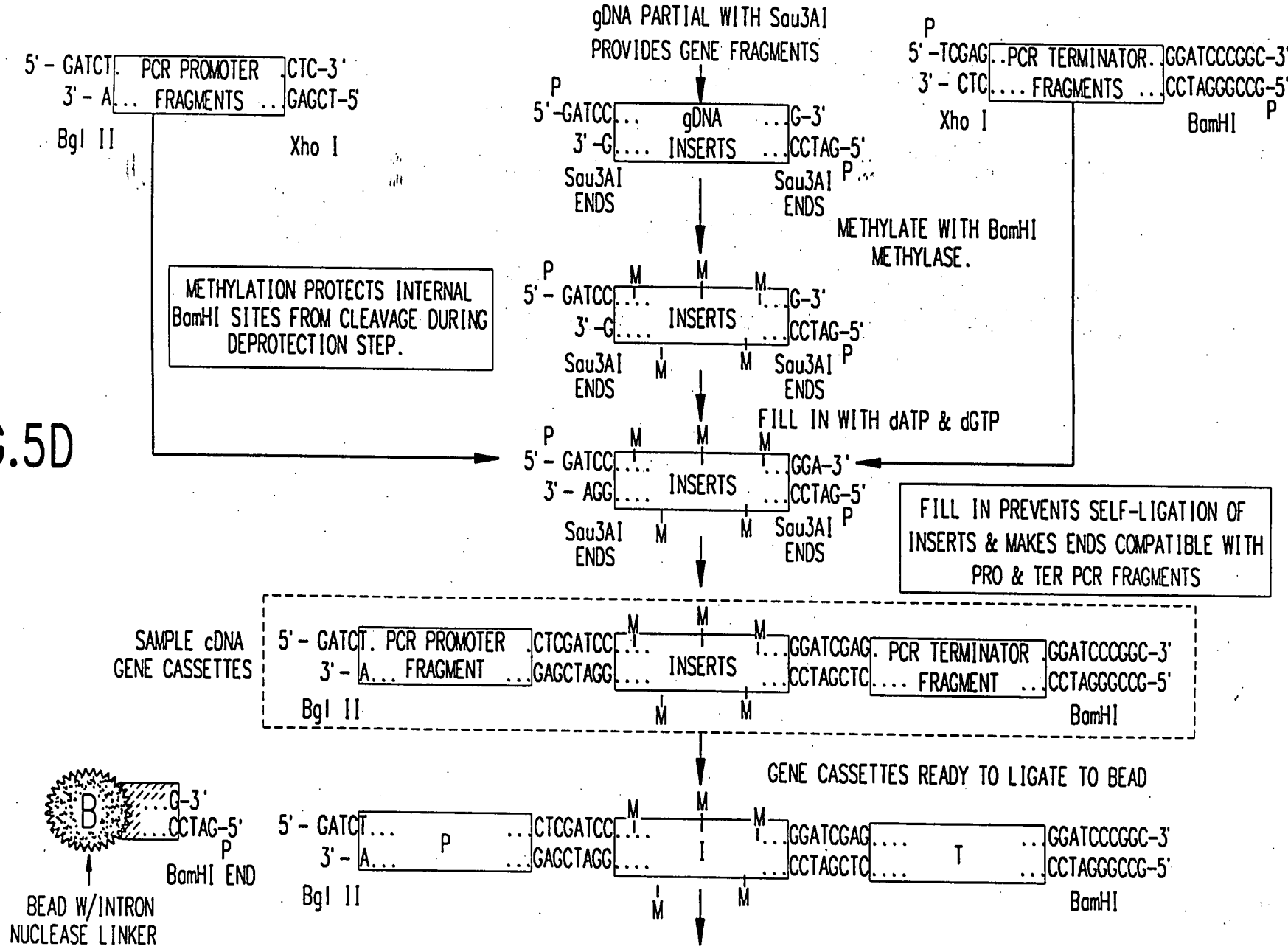
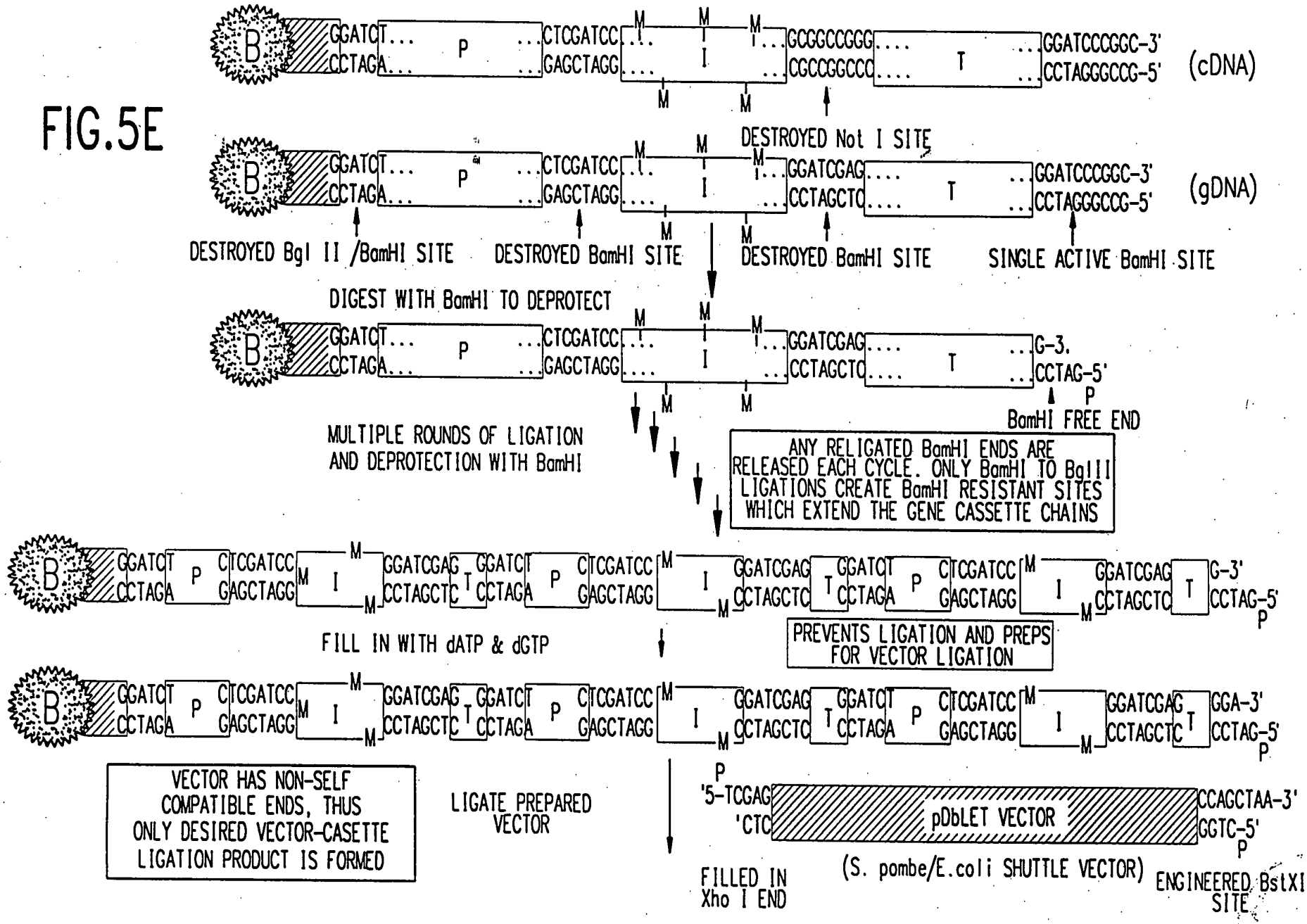

gDNA PARTIAL WITH Sau3AI
PROVIDES GENE FRAGMENTS
5' - GATCT PCR PROMOTER CTC-3'
3' - A FRAGMENTS GAGCT-5'
Bgl II
Xho I
P
5'-TCGAG PCR TERMINATOR GGATCCCGGC-3'
3' - CTC FRAGMENTS CCTAGGGCCG-5'
Xho I
BamHI
P
5'-GATCC gDNA G-3'
3'-G INSERTS CCTAG-5'
Sau3AI ENDS
Sau3AI ENDS
METHYLATE WITH BamHI METHYLASE.
METHYLATION PROTECTS INTERNAL BamHI SITES FROM CLEAVAGE DURING DEPROTECTION STEP.
M
5' - GATCC G-3'
3'-G INSERTS CCTAG-5'
FILL IN WITH dATP & dGTP
5' - GATCC GGA-3'
3' - AGG INSERTS CCTAG-5'
FILL IN PREVENTS SELF-LIGATION OF INSERTS & MAKES ENDS COMPATIBLE WITH PRO & TER PCR FRAGMENTS
SAMPLE cDNA GENE CASSETTES
5' - GATCT PCR PROMOTER CTCGATCC GGATCGAG PCR TERMINATOR GGATCCCGGC-3'
3' - A FRAGMENT GAGCTAGG CCTAGCTC FRAGMENT CCTAGGGCCG-5'
Bgl II
BamHI
GENE CASSETTES READY TO LIGATE TO BEAD
B
G-3'
CCTAG-5'
P
BamHI END
BEAD W/INTRON NUCLEASE LINKER
5' - GATCT P CTCGATCC I GGATCGAG T GGATCCCGGC-3'
3' - A GAGCTAGG CCTAGCTC CCTAGGGCCG-5'
Bgl II
BamHI

FIG.5E



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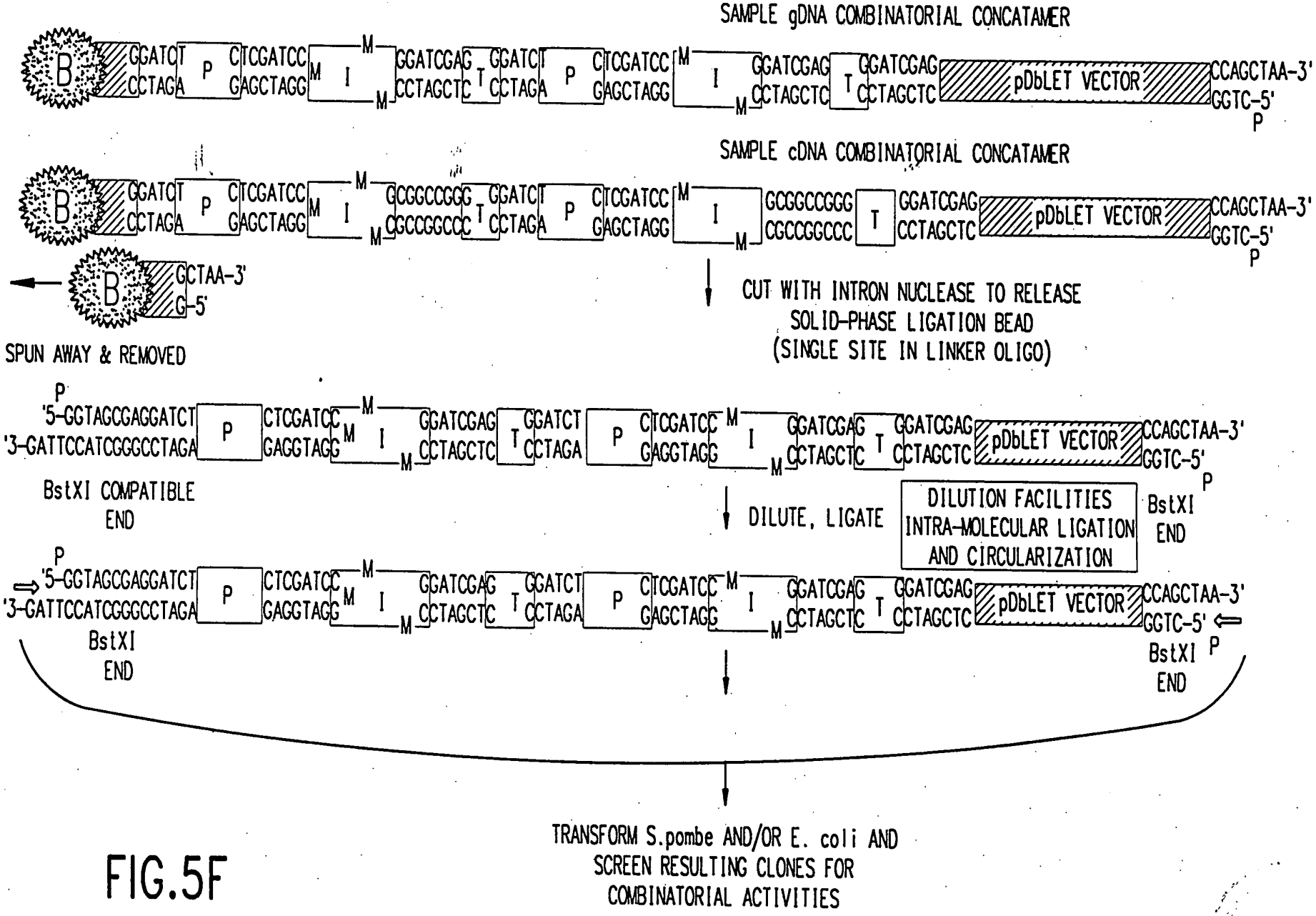

SAMPLE gDNA COMBINATORIAL CONCATAMER
SAMPLE cDNA COMBINATORIAL CONCATAMER
pDbLET VECTOR
CUT WITH INTRON NUCLEASE TO RELEASE
SOLID-PHASE LIGATION BEAD
(SINGLE SITE IN LINKER OLIGO)
SPUN AWAY & REMOVED
BstXI COMPATIBLE END
DILUTE, LIGATE
DILUTION FACILITIES
INTRA-MOLECULAR LIGATION
AND CIRCULARIZATION
BstXI END
TRANSFORM S.pombe AND/OR E. coli AND
SCREEN RESULTING CLONES FOR
COMBINATORIAL ACTIVITIES

FIG.5F

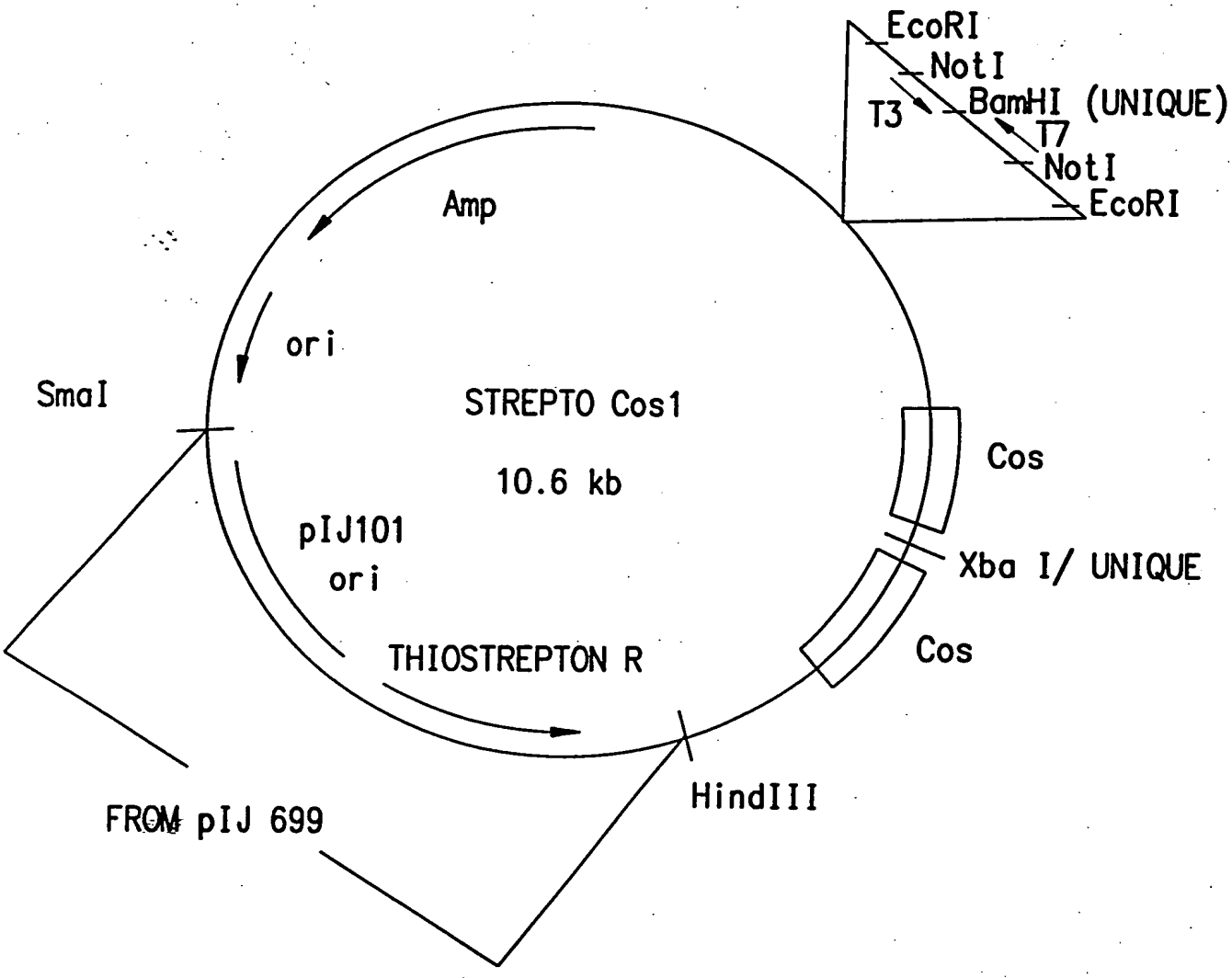
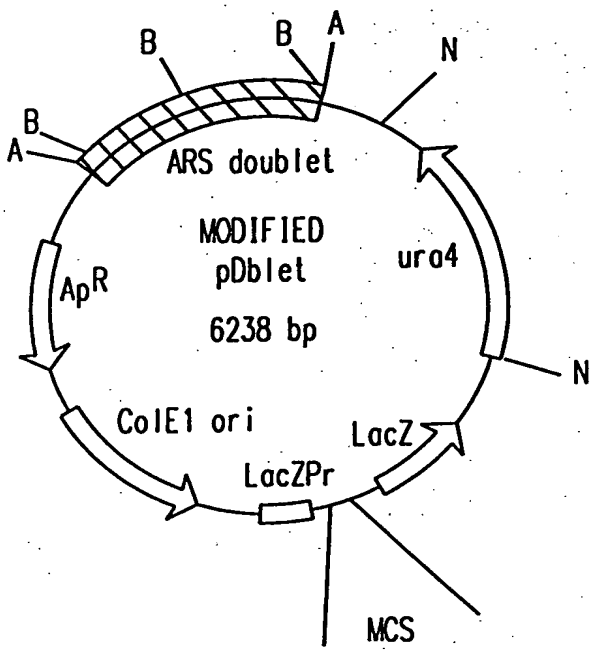

EcoRI
NotI
T3
BamHI (UNIQUE)
T7
NotI
EcoRI
Amp
ori
SmaI
STREPTO Cos1
10.6 kb
Cos
pIJ101
ori
Xba I/ UNIQUE
THIOSTREPTON R
Cos
HindIII
FROM pIJ 699

FIG.6A



MCS = SacI-NcoI-BstXI-NotI-Xba...

FIG.6B

5' CCTAGCCATGGCCACCTAACTGGGATCGC 3'

3' TCGAGGATCGGTACCGGTGGATTGACCCTAGCGCCGG 5'

SacI NcoI BstXI NotI END

FIG.6C

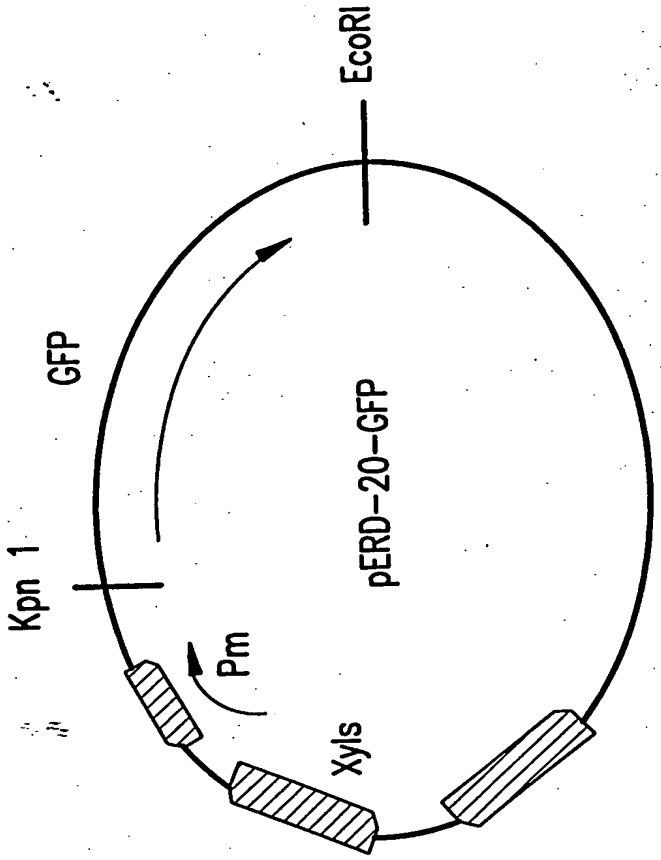


FIG.7

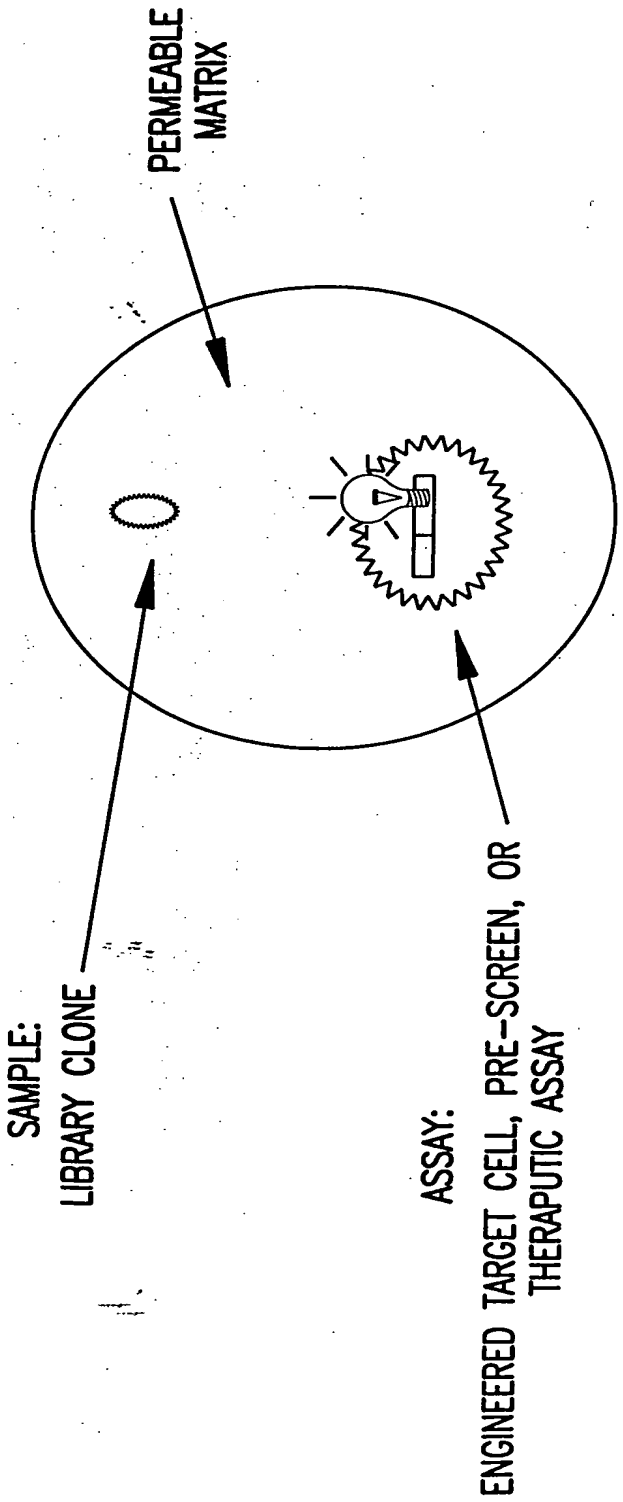

PERMEABLE MATRIX
SAMPLE: LIBRARY CLONE
ASSAY: ENGINEERED TARGET CELL, PRE-SCREEN, OR THERAPUTIC ASSAY
MACRODROPLET

FIG.8

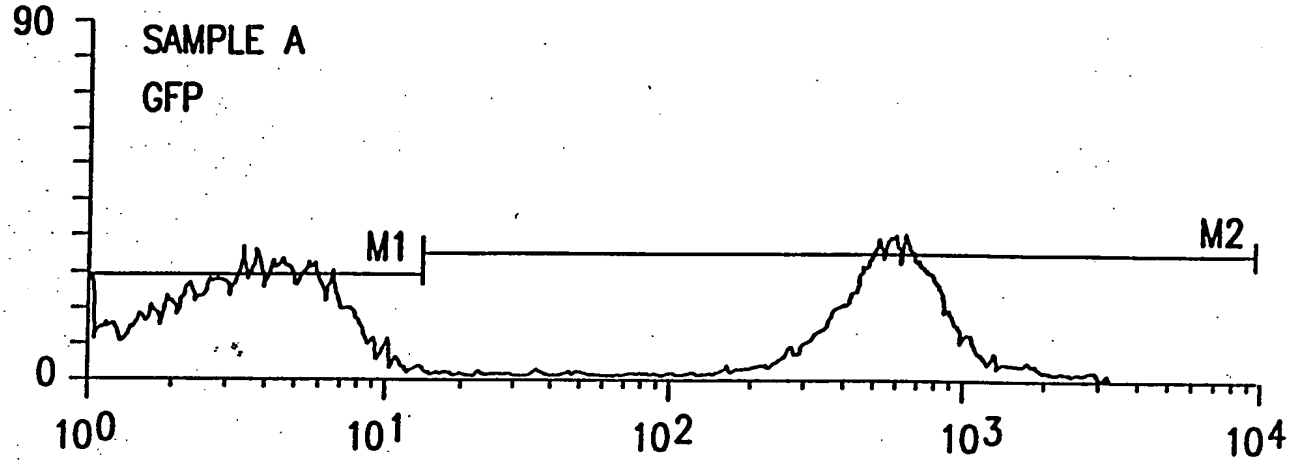

90
SAMPLE A
GFP
M1
M2
0
10^0
10^1
10^2
10^3
10^4

FIG.9A

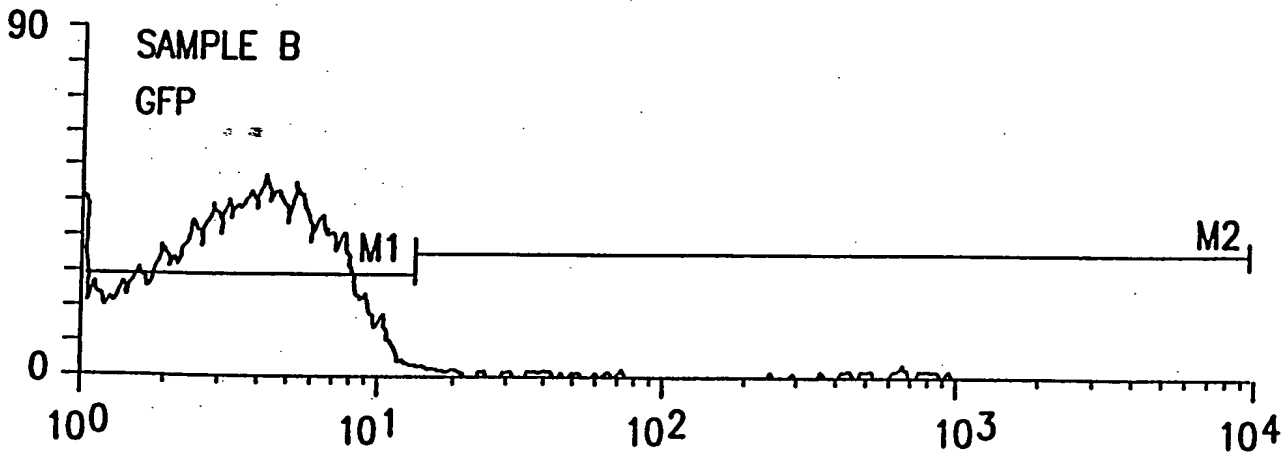

90
SAMPLE B
GFP
M1
M2
0
10^0
10^1
10^2
10^3
10^4

FIG.9B

```
                           POSITION
           CXC-AMN20       (1)  - - - - - - - - - - - - - - - - - - - - - - - - - N S H F I I T T Q G V L V F D S G S S E H I G N A  (23)
ACTINORHODIN DEHYDRASE       1  M T V E V R E V A E G V Y A Y E Q A P G G W C V S N A G I V V G G D G A L V V D T L S T I P R A R R  50

           CXC-AMN20      (24)  I I A A V K R V T E Q P I R W V V N S H S H A D H W L G N A A L A K L G A E L I S T S L S A E T M K  (73)
ACTINORHODIN DEHYDRASE      51  L A E W V D K L A A G P G R T V V N T H F H G D H A F G N Q V F A P - G T R I I A H E D M R S A M V  99

           CXC-AMN20      (74)  S D G P V D V K A F F N M T K G A T G E S T L V I P T S I I L H Q Q T R T F G D T E V E F V F A N D  (123)
ACTINORHODIN DEHYDRASE     100  T T G L A L T G - L W P - - R V D W G E I E L R P P N V T F R D R L T L H V G E R Q V E L T C V G P  146

           CXC-AMN20     (124)  G H S P G D V M L W L P K Q R I L I G G D V V N S N F M P I M T P R G N I T Q L I S V L K E V E Q L  (173)
ACTINORHODIN DEHYDRASE     147  A H T D H D V V V W L P E E R V L F A G D V V M S G V T P - F A L F G S V A G T L A A L D R L A E L  195

           CXC-AMN20     (174)  S P L L V L T G H G E N T S V K S V S R D I Q F L T Y A S N A V H E A L V K G T T P A K I Q A S L Q  (223)
ACTINORHODIN DEHYDRASE     196  E P E V V V G G H G P V A G P E V I D A N R D Y L R W V Q R L A A D A V D R R L T P L Q A A R R A D  245

           CXC-AMN20     (224)  A T L R T K F G K A Y Q D F D T S I S Y L L E M M I D K Q R L Q F S P I T - - - - - - - - - - - - - -  (264)
ACTINORHODIN DEHYDRASE     246  L G A F A G L L D A E R L V A N L H R A H E E L L G G H V R D A M E I F A E L V A Y N G G Q L P T C  295

           CXC-AMN20         -  - -                                                                                                 -
ACTINORHODIN DEHYDRASE     296  L A *                                                                                               297
```

FIG.10

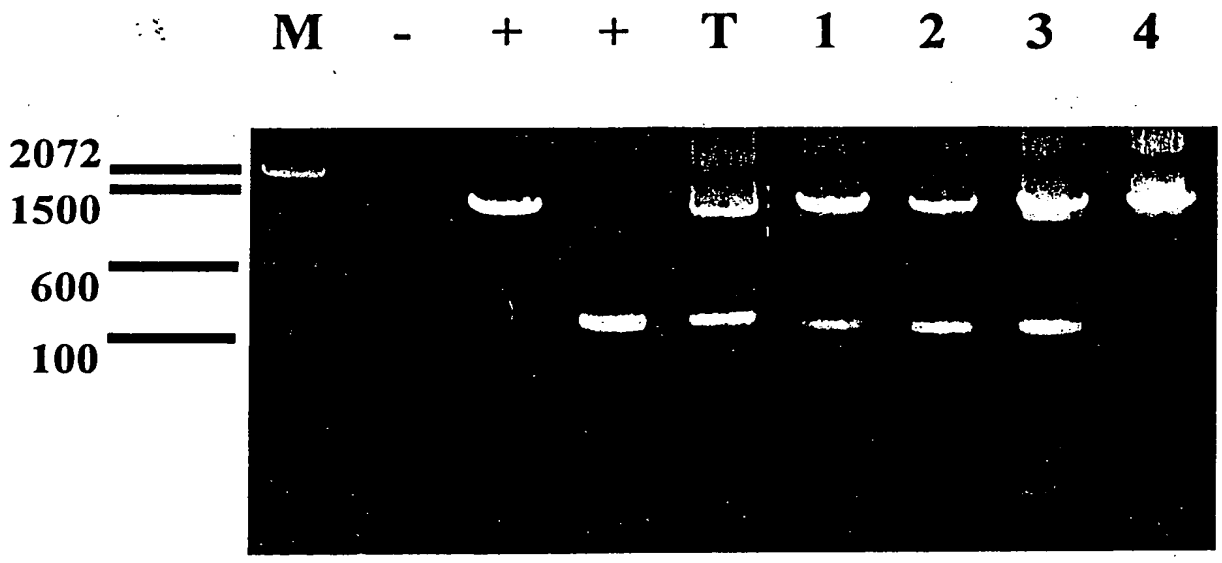


FIG. 11

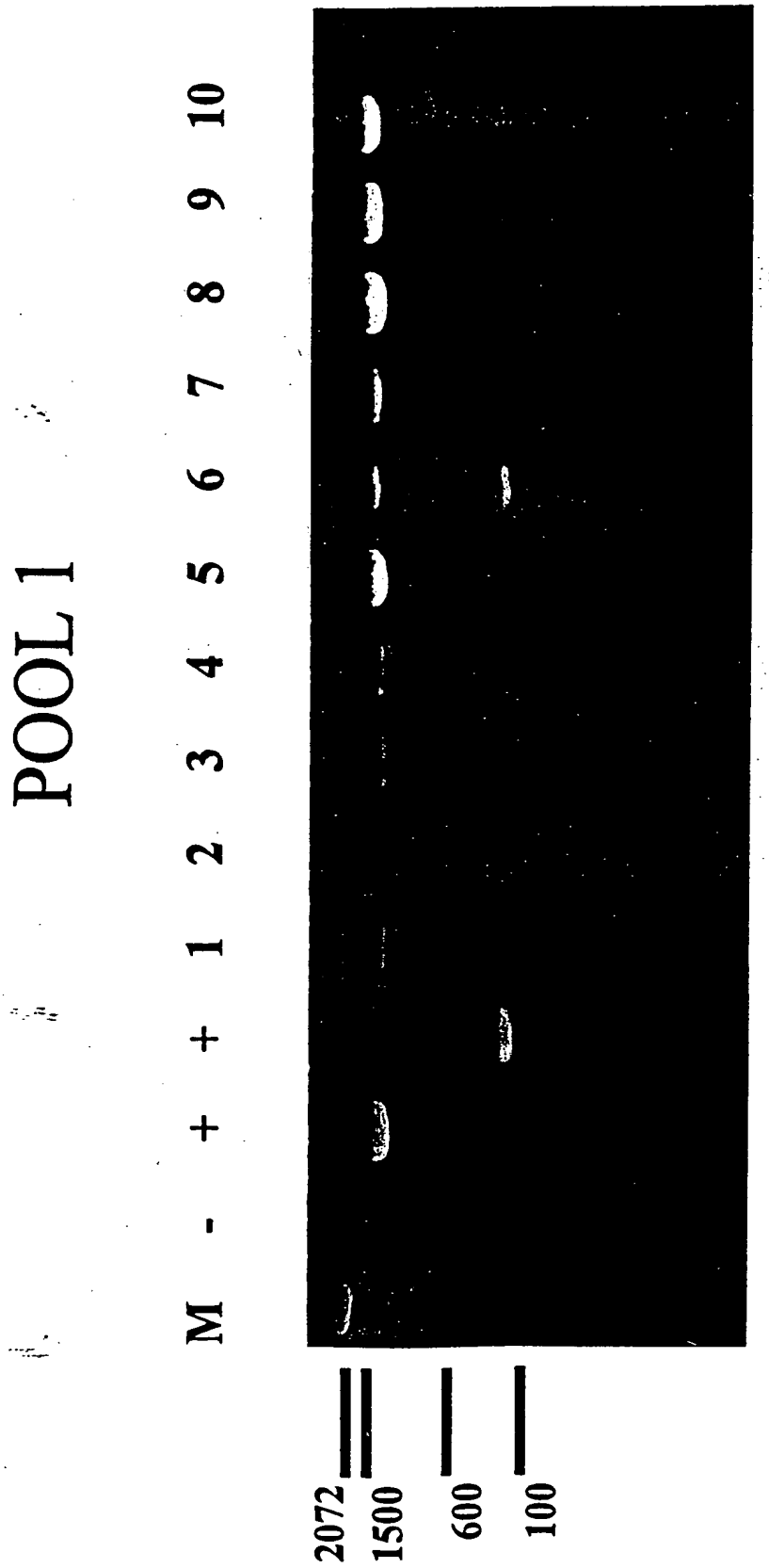


FIG.12A

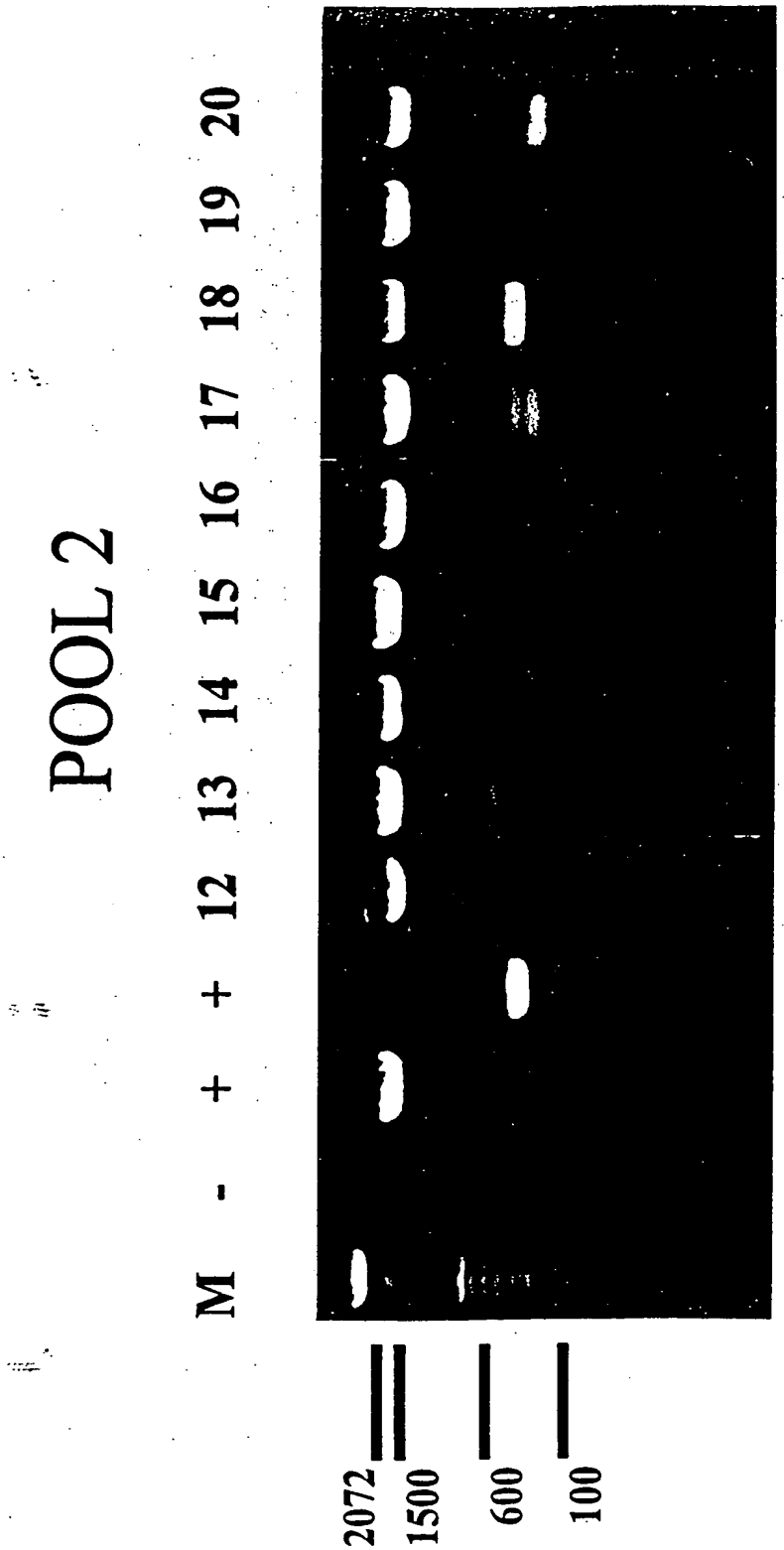


FIG.12B

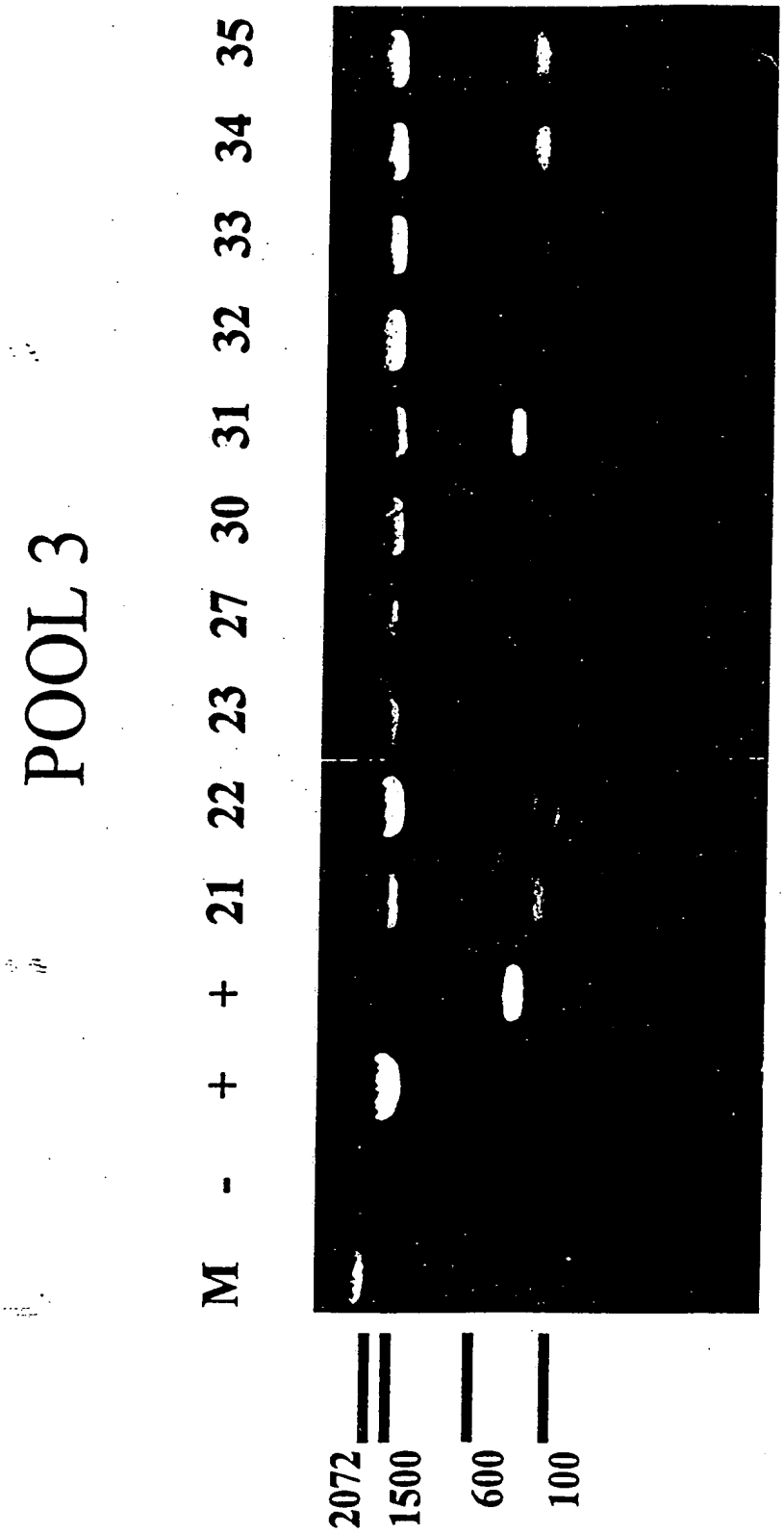


FIG.12C

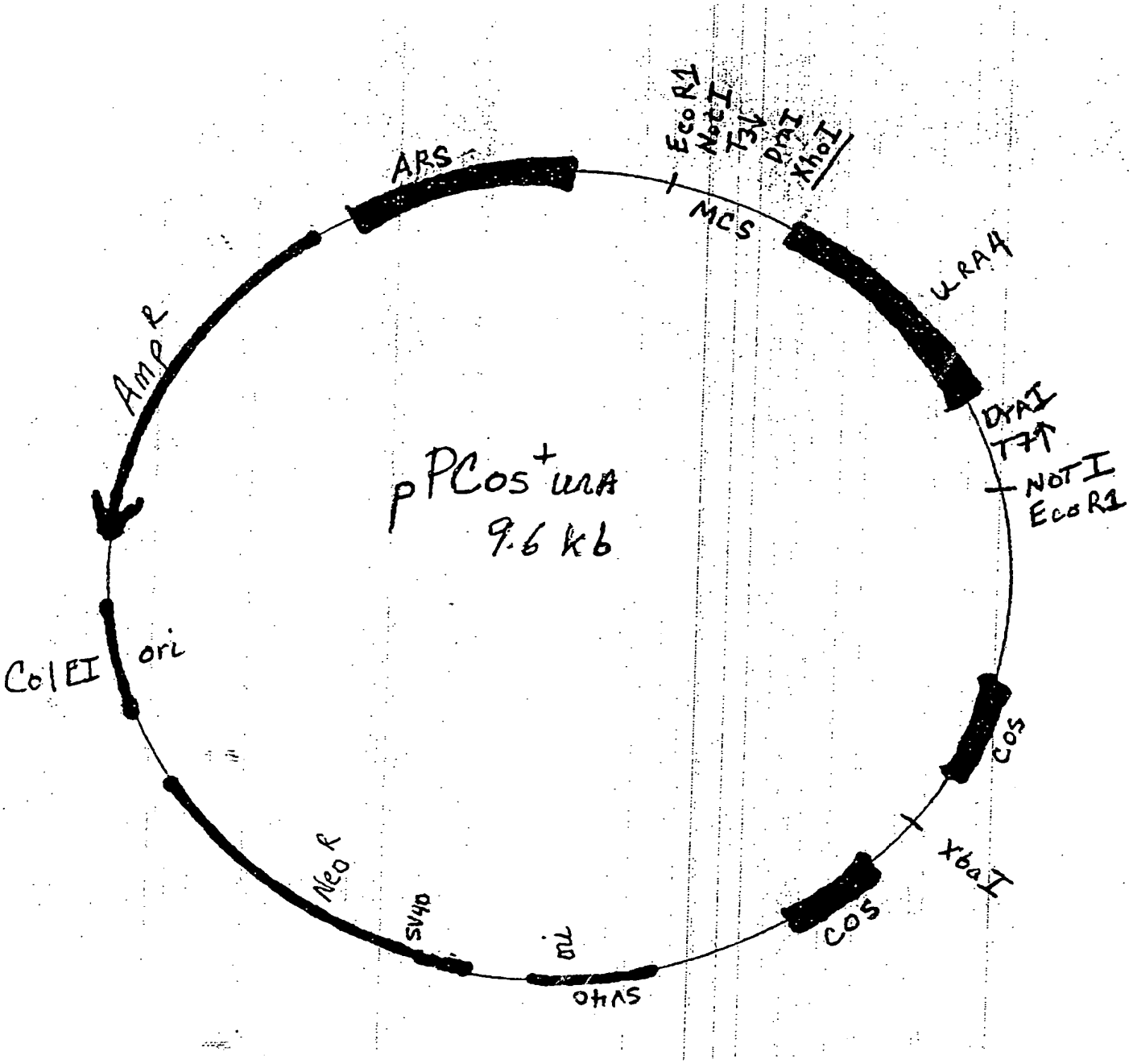

ARS
EcoR1
NotI
T3
DYAI
XhoI
MCS
URA4
DYAI
T7
NOTI
EcoR1
COS
XbaI
COS
SV40
pA
Neo R
ColEI ori
Amp R
pPCos+ura
9.6 kb

FIG. 13

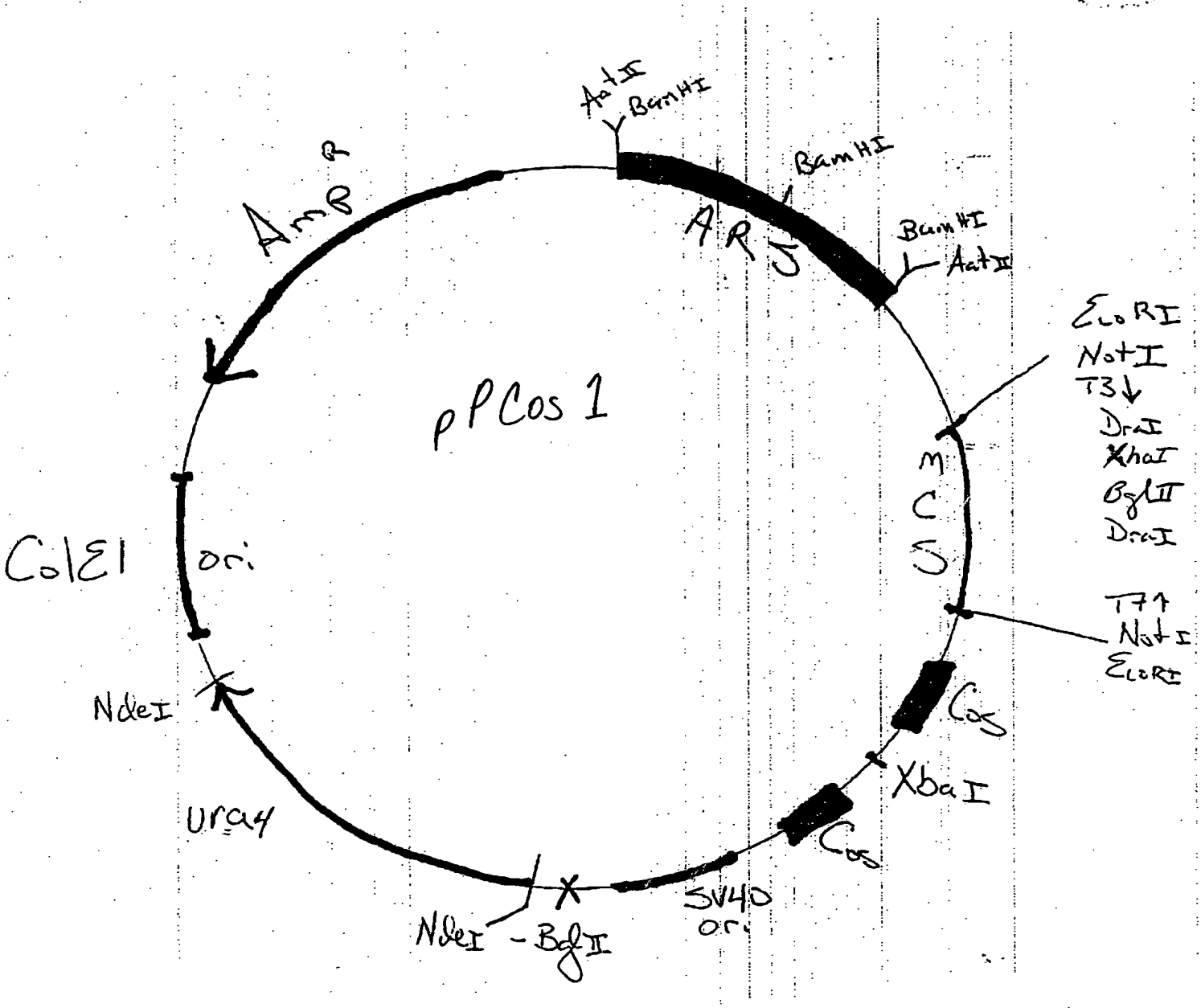

AatII
BamHI
BamHI
BamHI
AatII
AmpR
ARS
pPCos 1
EcoRI
NotI
T3
DraI
XhoI
BglII
DraI
MCS
T7
NotI
EcoRI
Cos
XbaI
Cos
SV40 ori
NdeI - BglII
URA4
NdeI
ColE1 ori

FIG. 14